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Borrelia burgdorferi sensu lato infection pressure shapes innate immune gene evolution in natural rodent populations across Europe

Barbara Tschirren

Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

Contact Information: barbara.tschirren@ieu.uzh.ch

Running title: *Borrelia* shapes *TLR2* evolution

Abstract

Although parasite-mediated selection is assumed to be the main driver of immune gene evolution, empirical evidence that parasites induce allele frequency changes at host immune genes in time and / or space remains scarce. Here I show that the frequency of a protective gene variant of the innate immune receptor Toll-like receptor 2 (*TLR2*) in natural bank vole (*Myodes glareolus*) populations is positively associated with the strength of *Borrelia burgdorferi* sensu lato infection risk across the European continent. Thereby, this study provides rare evidence for the role of spatially variable infection pressures in moulding the vertebrate immune system.

Keywords: host-parasite interactions; Lyme disease; wildlife disease; genetic variation; resistance evolution; rodents

1. Introduction

Parasites negatively affect host fitness and thereby select for host responses that prevent or reduce infection. The immune system has a key role in host defence, and parasites are therefore assumed to be main drivers of immune gene evolution [1]. Associations between immune gene variants and parasite resistance are regularly observed in natural populations [2-4]. However, in most cases it is difficult to track changes in allele frequencies over time to directly demonstrate parasite-driven evolution of the hosts' immune system in the wild [5].

As an alternative, spatial variation in parasite abundance might provide insights into the role of infection pressures in shaping immune system evolution. In humans, for example, spatial variation in *Plasmodium* sp. prevalence is a strong predictor of the frequency of malaria resistance alleles across countries [6-8]. Evidence for an association between parasite pressure and the frequency of resistance alleles in populations of non-human vertebrates, however, remains scant because of a lack of information on spatial variation in the prevalence of wildlife disease, but also, or mainly, the scarcity of candidate immune genes [9].

Borrelia burgdorferi sesu lato is a common tick-transmitted pathogen in rodents [10], and the causative agent of human Lyme borreliosis (LB), the most common vector-borne disease in Europe and North America [11, 12]. The innate immune receptor Toll-like receptor 2 (*TLR2*) plays a key role in the recognition of bacterial lipoproteins [13] and has been identified as a candidate gene for *Borrelia* resistance in laboratory mice [14, 15].

Furthermore, bank voles (*Myodes glareolus*) carrying certain *TLR2* variants (*TLR2* c_2 [16]) have a substantially reduced probability of becoming *Borrelia* infected (i.e. *TLR2* c_2 confers partial *Borrelia* resistance), highlighting that *TLR2* mediates host-*Borrelia* interactions also in the wild [16]. Based on this knowledge, I hypothesised that *Borrelia*-mediated selection shapes the evolution of *TLR2* in rodents. Indeed, substantial variation in the frequency of the protective *TLR2* variant has been observed in bank vole populations across Europe [17]. Although, data on *Borrelia* prevalence in these populations are not available, rates of human LB cases are reported for most European countries [18], and can be used as a proxy for *Borrelia* infection risk.

2. Material and methods

Information on Lyme borreliosis (LB) incidence in a country, measured as the average annual number of LB cases per 100'000 inhabitants, was obtained from [18] and [19] (Table 1). Data on the frequency of the protective *TLR2* c_2 variant in the local bank vole population were obtained from [17] (Table 1). Eight well-defined mitochondrial bank vole lineages have been described in Europe [20-22], which reflect the colonisation history of the species after the last glaciation. Phylogeographic analyses based on the mitochondrial cytochrome *b* gene were used to identify to which lineage the different study populations belong [17] (Table 1). For the UK, Germany and Italy two populations were sampled (Table 1). However, the results did not change qualitatively when randomly excluding one of the two populations per country from the analysis.

77 The frequency of *TLR2* c_2 in the sampled bank vole populations was analysed
78 using a quasibinomial generalised linear model in R [23]. LB incidence in a
79 country was included in the model. In addition, I included the mitochondrial
80 lineage to which a population belongs to account for potential differences in
81 *TLR2* c_2 frequency among populations due to the colonisation history of bank
82 voles in Europe [17, 20-22]. The significance of factors was determined by
83 comparing two nested models, with and without the factor of interest, using
84 likelihood-ratio tests.
85
86 3. Results and discussion
87 As predicted if *Borrelia*-mediated selection affects the large-scale
88 geographical distribution of the protective *TLR2* variant, there was a positive
89 association between the frequency of *TLR2* c_2 in bank vole populations and
90 human LB incidence across 19 European countries (χ^2 = 35.636, *DF* = 1, *P* =
91 0.003; Fig. 1). No indication was found that *TLR2* c_2 frequency differed among
92 mitochondrial bank vole lineages (χ^2 = 13.958, *DF* = 4, *P* = 0.499), showing
93 that the observed pattern was not influenced by the colonisation history of the
94 species in Europe [17, 20-22]. Rather, the strong association between human
95 LB incidence and the frequency of the protective *TLR2* c_2 variant in bank vole
96 populations indicates that *Borrelia*-mediated selection has shaped the
97 evolution of this innate immune receptor in wild rodents.
98 Clearly, the average annual rate of human LB cases in a country is
99 only a crude estimate of the actual *Borrelia* exposure bank voles experience
100 at a local scale. Lyme borreliosis incidence rates, for example, show regional
101 differences within countries [18]. Also, there might be differences among

countries in how accurately LB cases are diagnosed and / or reported [18]. Furthermore, lifestyle differences across countries might change the relationship between *Borrelia* abundance and human LB incidence [18]. Finally, human LB cases are partly caused by *Borrelia* genospecies that do not infect rodents (e.g. the bird specialist *Borrelia garinii* [24]). However, all these factors will add noise to the data and therefore reduce the statistical power to detect a relationship between human LB incidence and the frequency of the protective *TLR2* variant in the local bank vole population, rather than generate a spurious correlation. The observed relationship thus likely represents an underestimation of the impact of *Borrelia* pressure on *TLR2* evolution in bank voles.

The finding that the protective *TLR2* c_2 variant occurred at low frequencies in regions where *Borrelia* is rare suggests that resistance may be associated with costs in the absence of the pathogen [25-27]. Indeed, it has been shown that a human Toll-like receptor 4 (*TLR4*) variant reduces mortality during malaria infection, but is disadvantageous in the absence of *Plasmodium* because it increases the susceptibility to severe bacterial infections [7]. Similar trade-offs may maintain the pronounced balanced polymorphism observed at the bank vole *TLR2* [17]. Assessing the costs of carrying resistance alleles in the absence of infection will thus be a fruitful next step to understand the maintenance of immunogenetic variation in wild vertebrates.

In conclusion, this study provides empirical evidence for an association between *Borrelia* infection risk and the frequency of a protective *TLR2* variant in bank vole populations across the European continent. Thereby it is one of

the first to reveal an association between large-scale spatial variation in pathogen pressure and the immunogenetic composition of populations of a wild vertebrate.

Data accessibility

All data supporting this article are presented in Table 1.

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Conflict of interests

I have no competing interests.

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234

235 Table 1 Frequency of the protective *TLR2* c_2 variant (*TLR2*c_2) in bank vole
236 populations across Europe and average number of annual Lyme borreliosis
237 cases per 100'000 inhabitants (*LB incidence*) in the same country. LB
238 incidence data were obtained from [18] and [19]. *N*: number of sequenced
239 bank voles; *Lineage*: mitochondrial lineage of the local bank vole population.
240 The exact bank vole sampling locations and *TLR2 and cytb* genotyping
241 procedures are described in [17].

242

Country	***N***	***TLR2c₂***	***Lineage***	***LB incidence***
Austria	17	0.27	Western	130
Belgium	19	0.05	Western	12.58
Czech Republic	19	0.00	Western	31.73
Denmark	19	0.05	Eastern	1.68
England	17	0.00	Western	1.72
Finland	20	0.03	Eastern	18.46
Germany 1	21	0.10	Western	25
Germany 2	18	0.08	Eastern	25
Italy 1	21	0.00	Italian	0.02
Italy 2	19	0.00	Italian	0.02
Lithuania	20	0.18	Carpathian	42.93
Netherlands	10	0.00	Western	2.01
Norway	21	0.00	Carpathian	4.5
Poland	12	0.29	Eastern	9.29
Russia	21	0.07	Eastern	9.24
Scotland	19	0.00	Carpathian	1.72
Slovenia	20	0.30	Western	136.86
Spain	13	0.00	Spanish	9.8
Switzerland	19	0.27	Western	25.09
Sweden	20	0.30	Carpathian	69
Ukraine	20	0.00	Eastern	0.98

Figure legend

Figure 1. Relationship between human Lyme borreliosis incidence (LB cases / 100'000 inhabitants) and the frequency of the protective *TLR2* c_2 variant in the local bank vole population across 19 European countries. The circle size indicates the number of sampled *TLR2* alleles (*N*) in a population. Locally fitted smoothed polynomial surface (solid line) and 95% confidence interval (grey area) are presented.